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(71)(72) Applicants and Inventors: TSCHESCHE, Harald [DE/DE]; Frölenberg 39, D-33647 Bielefeld (DE). KRUMME, Dirk [DE/DE]; Frölenberg 39, D-33647 Bielefeld (DE).

(74) Agents: BOETERS, Hans et al.; Boeters & Bauer, Bereiteranger 15, D-81541 München (DE).

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(57) Abstract

Novel peptides containing the sequence -Pro-Leu-Ama(NHOH)- were synthesized and characterized by spectroscopic techniques. Their inhibitory properties towards the activated form of native human gelatinase B (MMP-9) and the catalytic domain of neutrophil colagenase (cdMMP-8) were determined. The most effective inhibitor synthesized exhibits K_i values of $2x10^{-6}$ M (cdMMP-8) and $5x10^{-9}$ M(MMP-9) thus attaining interesting discrimination between the tested metalloproteinases. A most important feature of this type of inhibitor is its peptide nature making the compounds similar to natural substrates. In spite of the peptide character of the inhibitors synthesized, the P_1-P_1'-peptide bond shows a high resistance to cleavage by the proteinases.

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MATRIX METALLOPROTEINASE INHIBITORS CONTAINING AMINOMALONIC ACID DERIVATIVES AND PEPTIDE BACKBONE MODIFIED DERIVATIVES THEREOF

The present invention relates to compounds being or containing aminomalonic acid derivatives and peptide backbone modified derivatives thereof acting as inhibitors of matrix metalloproteinases and thus being useful for the preparation of medicaments for treating pathophysiological processes connected to extracellular matrix disintegration.

Introduction and prior art.

Matrix metalloproteinases (MMPs) are a family of at least nineteen zinc-containing proteinases playing a fundamental role in the degradation and remodelling of connective tissue by hydrolysis of matrix proteins such as collagens, gelatins and proteoglycans (1). In this way MMPs are involved in many pathophysiological processes connected to extracellular matrix disintegration e.g. rheumatoid arthritis or tumor invasion and metastasis.

Matrix metalloproteinases have similar domain structures including as major domains an N-terminal pre-sequence, a prodomain, the catalytic domain and, in most cases, a hemopexin domain presumed to be involved in substrate specificity. The metalloproteinases are produced as zymogens and activated by removal of the prodomain e.g. by organomercuric agents or proteases. MMPs are divided into several subgroups based on their domain structures, sequence homologies and their substrate preferences.

Tissue inhibitors of metalloproteinases (TIMPs), naturally occurring proteins specifically inhibiting these proteinases, control MMPs in vivo. An imbalance between MMPs and their natural

antagonists, the TIMPs, can result in pathophysiological destructive processes such as rheumatoid arthritis, parodontosis or fibrosis. This may be compensated with potent synthetic inhibitors.

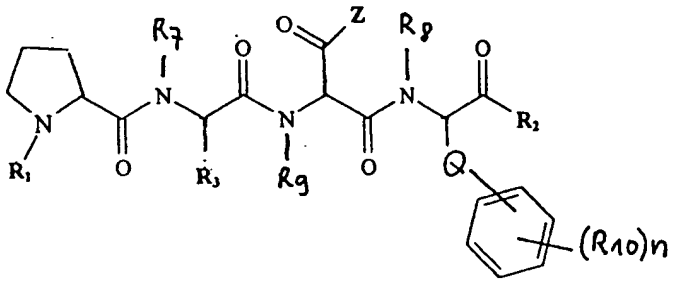
Numerous synthetic low-molecular weight MMP inhibitors have been synthesized (2, 3) and research is currently being carried out on their in vivo efficiency in different kinds of cancer or destructive joint diseases. Most of the synthetic MMP inhibitors developed so far are small molecular compounds binding to the catalytic site of the enzymes. Several X-ray crystal structures of the catalytic domains of collagenases complexed with an inhibitor have been published (4-7).

Effective inhibitors are equipped with a zinc chelator group and a peptide or non-peptide backbone mimicking a natural substrate. The hydroxamic acid function is a very good zinc-chelating group thus providing the most potent inhibitors of MMPs. In general the hydroxamic acid-based inhibitors are of the C-terminal type, binding at S´-subsites of the enzyme (nomenclature S_n ...S_n´, P_n ...P_n´ according to Schechter and Berger (8)). N-terminal inhibitors are much less effective. For the most part, C-terminal hydroxamic acid inhibitors are succinyl derivatives with various aliphatic or aromatic substituents on either the α- or β-carbon or both and residues at the γ-carboxyl function, attached by a peptide linkage.

An object of the invention is to provide a novel type of hydroxamate-based peptide inhibitors of MMPs.

Accordingly, the present invention provides compounds containing aminomalonic acid derivatives and peptide backbone modified de-

rivatives thereof of the general formulas I, II and III, IV, V and VI:



(I)

wherein

R_1 represents a N-protecting group like tert.butyloxycarbonyl, benzyloxycarbonyl or FMOC; or acetyl, -CO-lower alkyl, -CH_2-aryl, a natural amino acid, lower alkyl, aryl or H; or an optionally spacer linked: such as a synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

R_2 represents NH-D-C(Ph)-CH_3, NH-L-C(Ph)-CH_3, N(lower alkyl)$_2$, NH-lower alkyl, NH-aryl, a natural amino acid, a lower alkyl ester of an amino acid, O-lower alkyl, NHOH or OH, or an optionally spacer linked: such as a synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;
or R_4, wherein R_4 has the significance given below;
or the residue **Ccc**, wherein **Ccc** has the significance given below, optionally with a bounded residue R_2, wherein R_2 has the significance given earlier;
or **Z**, wherein **Z** has the significance given below;

R_3 represents lower alkyl or a side chain of a natural amino acid, or R_4;

R_7, R_8 and R_9 which may be the same or different from each other represent H, alkyl, aryl, OH, CO-lower alkyl, O-lower alkyl, O-CH_2-aryl, O-aryl, or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;
or an optionally spacer linked: such as synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

$(R_{10})_n$ (with n being selected from 0 to 5) which may be the same or different from each other are defined as R_2 or R_4 or $-(CH_2)_mR_4$ (with m being selected from 0 to 6);

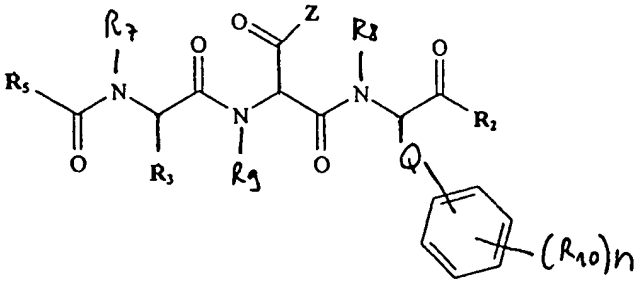
R_4 represents H, alkyl, aryl, OH, O-lower alkyl, O-CH_2-aryl, O-aryl, NH-CO-aryl, NH-CO-NH-aryl, NH-CO-CH_2-aryl or NH-CO-R_5, wherein R_5 has the significance given below;
NH_2, NH-lower alkyl, N(lower alkyl)$_2$, N(lower alkyl 1)(lower alkyl 2), NHaryl, N(aryl)$_2$, N(aryl 1)(aryl 2), also combinations and pharmaceutically acceptable salts thereof;
N(lower alkyl)$_3^+$, N(aryl)$_3^+$, also combinations thereof;
or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;

or an optionally spacer linked: such as synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

Z represents OH, O-lower alkyl, NHOH, $N(CH_3)OH$, $NHO-CH_3$, other NHO-lower alkyl,

Q represents $-(CH_2)_m-$, $-O-(CH_2)_m-$, $-CO-(CH_2)_m-$, $-(CH_2)_m-P-$, $-O-(CH_2)_m-P-$, $-CO-(CH_2)_m-P-$ (with m being selected from 0 to 6), wherein **P** represents cyclopropyl, cyclopentyl, cyclohexyl, 5- or 6-membered aryl, or a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and/or S as an additional ring member and (b) is optionally benzo-fused or optionally substituted on one or more other ring C-atoms by lower alkyl, aryl and/or oxo;
and pharmaceutically acceptable salts thereof; and

wherein
the term "lower alkyl", alone or in combination, means a straight-chain or branched-chain alkyl group containing a maximum of six carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert.butyl, n-pentyl, n-hexyl and the like;
the term "aryl" means phenyl, which is optionally substituted by lower alkyl, O-lower alkyl and/or halogen, i.e. fluorine, chlorine, bromine or iodine,
the term "spacer" means a straigth or branched alkyl-chain, amino alkyl-chain, carboxy alkyl-chain with a maximum length of 12 carbon atoms, or combinated forms, peptides or saccarides,



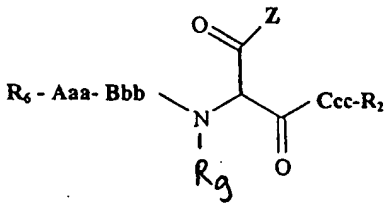
(II)

wherein R_2, R_3, R_7, R_8, R_9, R_{10}, Q, Z and n have the significance given earlier and

R_5 represents R_1-Proline, lower alkyl, aryl or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;

or an optionally spacer linked: synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

and pharmaceutically acceptable salts thereof;



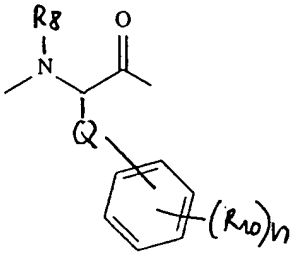
(III)

wherein R_2 , R_9 and Z have the significance given earlier and

Aaa represents a peptide bounded natural amino acid;

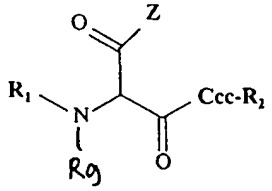
Bbb represents a peptide bounded natural amino acid;

Ccc represents a peptide bounded natural amino acid or Thr(Bzl),



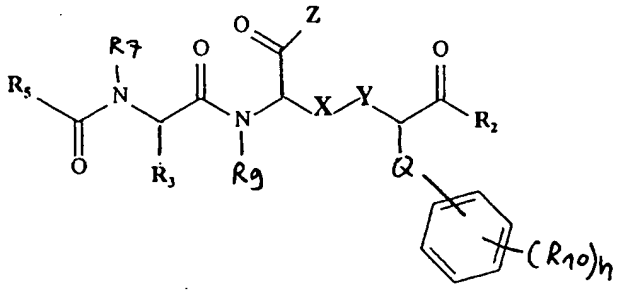
Ser(Bzl) or the fragment or $NR_8C(QR_{10})HCO$-, wherein R_8, R_{10}, **Q** and n have the significance given earlier;

R_6 represents a N-protecting group, acetyl, -CO-alkyl(C_1-C_4), a natural amino acid, lower alkyl or H or R_1, wherein R_1 have the significance given earlier;
and pharmaceutically acceptable salts thereof;



(IV)

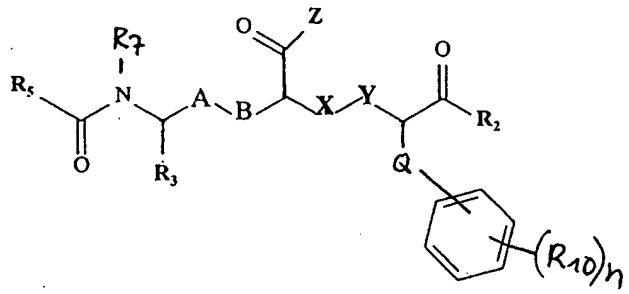
wherein R_1, R_2, R_9, **Z** and **Ccc** have the significance given earlier;
and pharmaceutically acceptable salts thereof;



V

wherein R_2, R_3, R_5, R_7, R_9, R_{10}, Q, Z and n have the significance given earlier,

X-Y represents especially CO-NH, CH_2-NH, CO-CH_2, CH_2-CH_2 CH_2-S, CH_2-O, CO-N(lower alkyl), CH_2-N(lower alkyl) or PHO_2-NH,
and pharmaceutically acceptable salts thereof;



VI

wherein R_2, R_3, R_5, R_7, R_{10}, Q, Z and n have the significance given earlier;

A-B and **X-Y** which may be the same or different from each other represent especially CO-NH, CH_2-NH, CO-CH_2, CH_2-CH_2 CH_2-S, CH_2-O, CO-N(lower alkyl), CH_2-N(lower alkyl)or PHO_2-NH;
and pharmaceutically acceptable salts thereof;
wherein any available hydrogen atom on any carbon or nitrogen atom in any of the above formulae I to VI and any of the corre-

sponding substituents or groups as defined for said formulae may be in part or totally and independently from each other substituted by halogen (bromine, chlorine, fluorine or iodine), alkyl, especially lower alkyl, aryl, OH, CO-lower alkyl, O-lower alkyl, O-CH_2-aryl, O-aryl, or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which (a) optionally contains N, O and/or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo.

Further, the compounds according to the general formulae **I, II, III, IV, V and VI** may have variations of the included chiral centers. All residues and chiral atoms of the compounds specified above may have the L- or D-conformation. Thus, any of the inventive compounds may be in the form of a L-enantiomer or a D-enantiomer or in the form of a diastereomer, including meso forms, or in the form of any mixture thereof, including racemic mixtures.

Further, the present invention provides the use of compounds according to general formulae **I, II, III, IV, V and VI** as inhibitors of metalloproteases.

Moreover, the present invention provides the use of compounds according to general formulae **I, II, III, IV, V and VI** as therapeutically active substances, especially in the control or prevention or in the treatment of:

degenerative joint diseases
rheumatoid arthritis
osteoarthritis
cancer

metastasis
tumour invasion
multiple sclerosis
parodontosis
fibrosis
Alzheimer´s desease
inflammatory bowel disease
neurodegenerative deseases
cerebral haemorrhage
wound healing
degenerative eye desease
aneurism
artificial joint replacement
organ transplantation
emphysema
Cholesteatom
Präeklampsie

Additionally, the present invention provides the use of compounds according to general formulae **I, II, III, IV, V and VI** in chromatographical procedures.

Detailed description of the invention with results and discussion.

In this invention a novel type of hydroxamic acid-based peptide inhibitor is presented. The residue carrying the hydroxamate function is an aminomalonic acid (Ama) in position P_1. This allows the addition of various residues suitable to gain maximum attachment to the entire binding site of the target enzyme on both S- and S´-subsides. The most important feature of this type of inhibitor is the peptide backbone built up by α-amino acids

to comprise the positions P_3- to P_1' at least, giving these compounds the same basic frame as a peptide substrate. In accordance with the specificity of the metalloproteinases tested the P_3-position of these inhibitors is occupied by a proline, P_2 is an aliphatic amino acid, whereby leucine or alanine is favored. The best inhibitory properties were obtained with the bulky tyrosine benzylether moiety in P_1'-position (Fig. 1).

The K_i values of the synthesized MMP inhibitors determined in quantitative fluorometric assays are shown in Table 1. Some of these compounds exhibit very good inhibitory properties against the enzymes tested. Analogous derivatives with free carboxyl function, methyl- or ethylesters at the aminomalonic acid instead of a hydroxamate in this position show virtually no inhibitory effect (9). Thus it is obvious that a zinc-complexing function is essential for a high affinity to the metalloproteinases. This observation confirms the assumption that these inhibitors bind competitively to the catalytic centre, with the hydroxamic acid in a proper position to chelate the zinc ion in the active site. The complexing group, representing the complete side chain of the amino acid in positon P_1, is fixed as close as possible to the peptide backbone and to the reactive-site bond.

Apart from this favorable conformational arrangement of the chelating group towards the metal ion another relevant criterion for effective inhibition is an optimal adjustment of the backbone to a natural substrate. The P_1-residue in a natural substrate is glycine. Its substitution by the aminomalonic acid derivative only adds a small chelating [illegible]nction to the [illegible]de. Except for the Ama(NHOH)-residue in P_1 position this type of peptide inhibitor is highly variable on both N- and C-terminal

sides, thus allowing designing of the rest of the structure for an optimal fit into to the enzyme subsites.

Corresponding to the substrate specificity of the tested metalloproteinases, the P_3-position of all inhibitors is occupied by a proline. A substitution of the proline in compound **1** by a Boc protecting group results in a decrease of the inhibition constant by about two orders of magnitude for the MMP-9 and one order for the cdMMP-8.

Compounds **5**, **6** and **7** with leucine at P_2-position have the strongest inhibitory effect toward the catalytic domain of MMP-8, in agreement with the sequences of various synthetic peptide substrates of collagenases (14-16). The inhibition constants of these compounds for the gelatinase B are about two orders of magnitude lower than those for the collagenase with its high substrate discrimination.

A more distinct selectivity between MMP-9 and cdMMP-8 is shown by compound **4** with alanine at the P_2-site (K_i = $5x10^{-9}$ M for MMP-9, $1.9x10^{-6}$ for cdMMP-8). Whereas the K_i value of **4** for the MMP-9 equals those of the analogous leucine derivatives, a distinct decrease can be observed for the cdMMP-8. There is a nearly 400-fold inhibitory discrimination of **4** between the two enzymes.

The glycine-containing derivates **1** to **3** inhibit the tested enzymes one to two orders of magnitude less efficiently. Thus an amino acid with a chiral center in position P_2 seems to be favorable. This effect is more pronounced for MMP-9, therefore these inhibitors are less selective.

The compounds with the bulky α-methylcyclohexanemethylamine at position P_2' have weaker inhibitory effects than the aromatic analogues.

Despite their peptide character the inhibitors were resistant to hydrolysis by the proteases as confirmed by HPLC investigations after incubation of the inhibitors with the metalloproteinases for several hours. None of the possible fragments from cleavage of the Ama-Tyr-bond could be detected. Thus, these inhibitors and especially the P_1-P_1'-peptide bond exhibit a high resistance to cleavage by the proteinases.

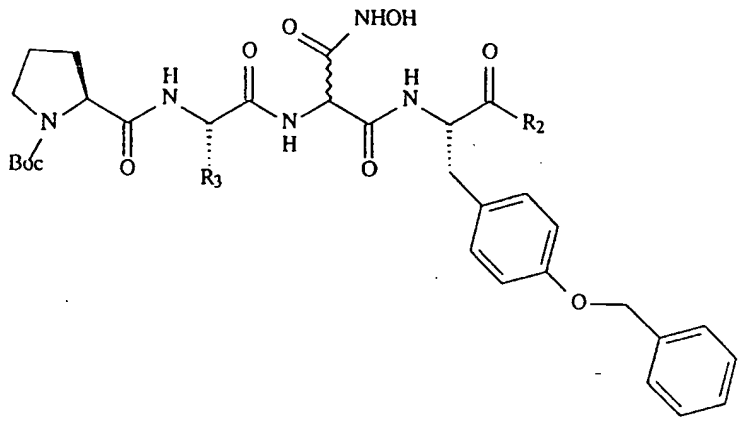
Figure and Table Legends

Fig. 1. Partial sequence of a typical substrate of gelatinase B (MMP-9) with the amino acid residues numbered according to Schechter and Berger (8) compared with the structure of an inhibitor of the type R_1-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-R_2 presumably binding at the subsites of the enzyme. R_1 is a protecting group, H, acetyl or an amino acid, R_2 is an amine, an alcohol, NHOH or a C-terminal protected amino acid or peptide.

Table 1. Tested inhibitors and their K_i values determined with gelatinase B (MMP-9) and the catalytic domain of the neutrophil collagenase (MMP-8) in mole/l. NH-R-CH(Ph)CH_3, NH-SCH(Ph)CH_3 and NH-R-CH(C_6H_{11})CH_3 are N-bounded residues of R(+)-α-methylbenzylamine, s(-)-α-methylbenzylamine, and R(-)-α-methylcyclohexanemethylamine.

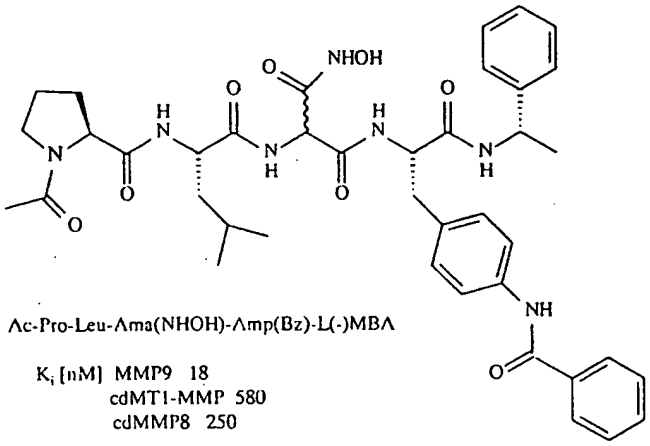
Abbreviations: Ama, aminomalonic acid; cdMMP, catalytic domain of an MMP, MMP, matrix metalloproteinase; DCC, dicyclohexyl carbodiimide; TIMP, tissue inhibitor of metalloproteinases.

Table 1.



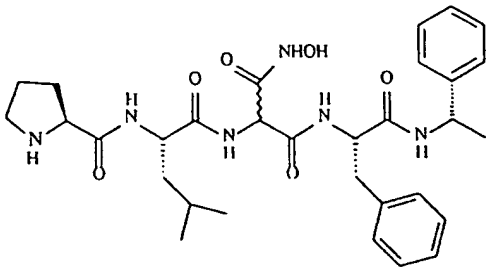
compound no.	R_2	R_3	MMP-9	cdMMP-8
1	NH-R-CH(Ph)CH_3	H	$4.0x10^{-7}$	$6.5x10^{-6}$
2	NH-S-CH(Ph)CH_3	H	$>10^{-7}$	$1.8x10^{-5}$
3	NH-R-CH(c-C_6H_{11})CH_3	H	$>10^{-7}$	$1.4x10^{-5}$
4	NH-S-CH(Ph)CH_3	CH_3	$5.0x10^{-9}$	$1.9x10^{-6}$
5	NH-R-CH(Ph)CH_3	CH_2-CH-$(CH_3)_2$	$5.0x10^{-9}$	$8.0x10^{-7}$
6	NH-S-CH(Ph)CH_3	CH_2-CH-$(CH_3)_2$	$5.0x10^{-9}$	$8.0x10^{-7}$
7	NH-R-CH(c-C_6H_{11})CH_3	CH_2-CH-$(CH_3)_2$	$1.8x10^{-8}$	$2.5x10^{-6}$

Further Examples with K_i-values (uncorrected) against some enzymes, respectively their catalytic domains (cd):



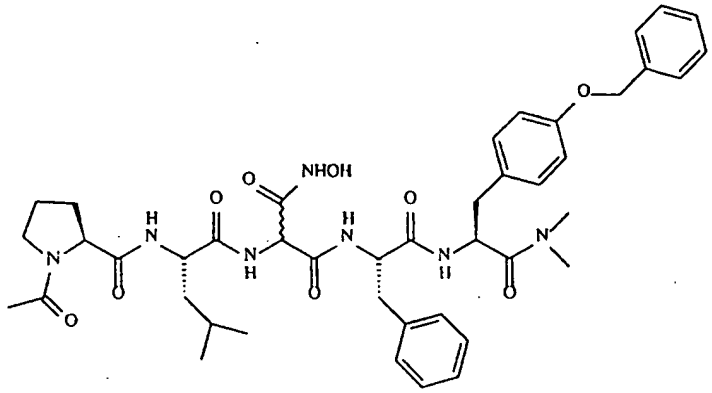
Ac-Pro-Leu-Ama(NHOH)-Amp(Bz)-L(-)MBA

K_i [nM] MMP9 18
cdMT1-MMP 580
cdMMP8 250



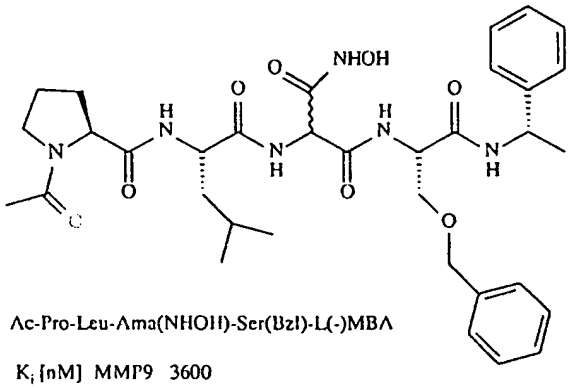
H-Pro-Leu-Ama(NHOH)-Phe-L(-)MBA

K_i [nM] cdMMP9 5000
cdMT1-MMP 6000
cdMMP8 10000



Ac-Pro-Leu-Ama(NHOH)-Phe-Tyr(Bzl)-NMe_2

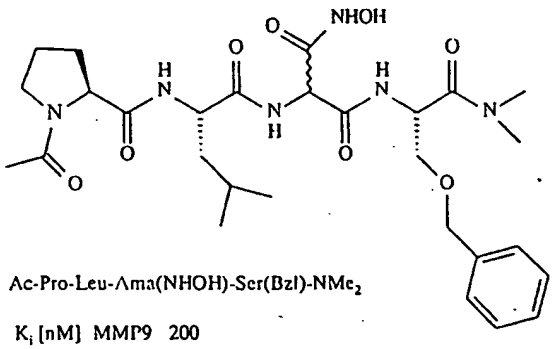
K_i [nM] MMP9 4,5



Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-L(-)MBA

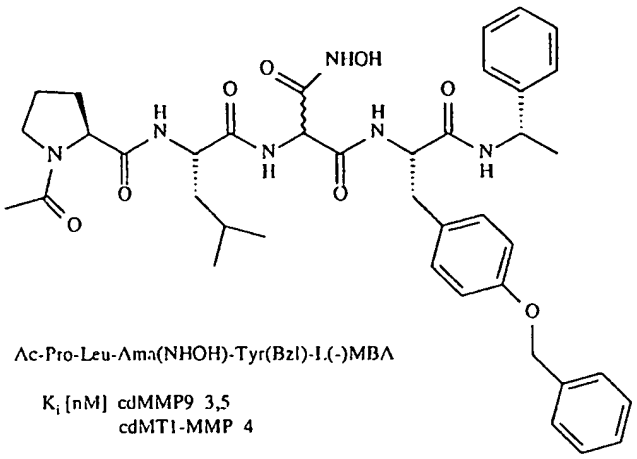
K_i [nM] MMP9 3600
cdMT1-MMP 1300
cdMMP8 710

5



Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-NMe_2

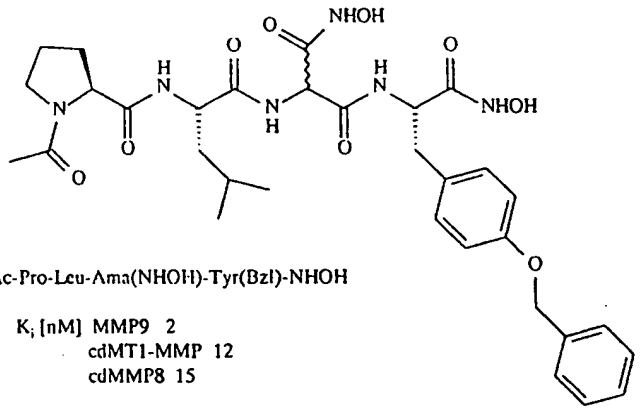
K_i [nM] MMP9 200
cdMT1-MMP 2200
cdMMP8 1000



Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-1.(-)MBA

K_i [nM] cdMMP9 3,5
cdMT1-MMP 4

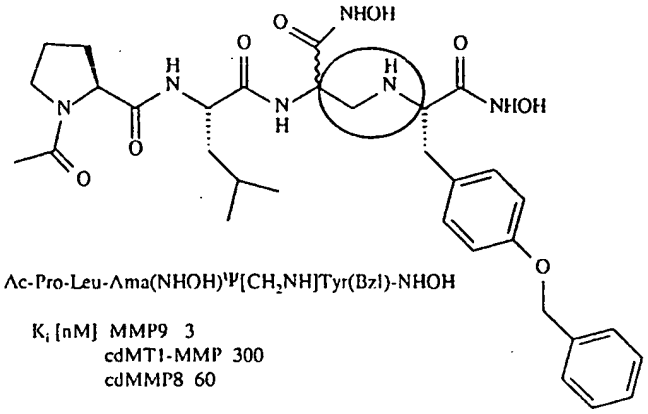
5



Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NHOH

K_i [nM] MMP9 2
cdMT1-MMP 12
cdMMP8 15

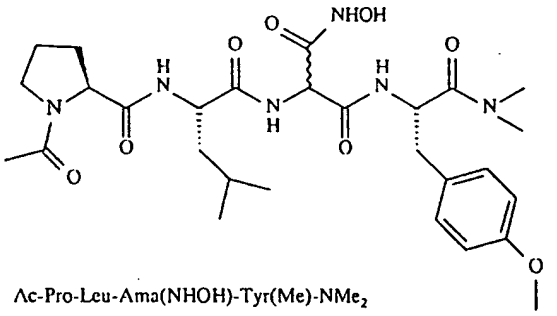
Analogous P_1P_1'-deoxopeptide:



Ac-Pro-Leu-Ama(NHOH)$^{\Psi}$[CH_2NH]Tyr(Bzl)-NHOH

K_i [nM] MMP9 3
cdMT1-MMP 300
cdMMP8 60

5



Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NMe_2

K_i [nM] MMP9 0,23
cdMT1-MMP 60
cdMMP8 200

Table 2: Preliminary K_i-values in nM (not corrected)

*) Structure see above

(cd = catalytic domain)

Inhibitor		MMP-9	cdMT1-MMP	cdMMP-8
Ac-Pro-Leu-Ama(NHOH)-Amp-L(-)MBA		1500	12000	2500
Ac-Pro-Leu-Ama(NHOH)-Amp(Bz)-L(-)MBA	*)	18	580	250
Ac-Pro-Leu-Ama(NHOH)-Amp(CONHPh)-L(-		35	3200	500
Ac-Pro-Leu-Ama(NHOH)-Amp(Z)-L(-)MBA		250	13000	90
Ac-Pro-Leu-Ama(NHOH)-Leu-2,4-DiMeP		2000	>10000	30000
Ac-Pro-Leu-Ama(NHOH)-2-Nal-NMe_2		8	1300	890
Ac-Pro-Leu-Ama(NHOH)-D-2-Nal-NHMe		76	5800	580
Ac-Pro-Leu-Ama(NHOH)-D-2-Nal-NMe_2		50	2700	100
H-Pro-Leu-Ama(NHOH)-Phe-D(+)MBA		6000		
H-Pro-Leu-Ama(NHOH)-Phe-L(-)MBA	*)	5000	6000	10000
Ac-Pro-Leu-Ama(NHOH)-Phe-Tyr(Bzl)-	*)	4,5		
Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-L(-	*)	3600	1300	710
Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-NMe_2	*)	200	2200	1000
Ac-Pro-Leu-Ama(NHOH)-Thr(Bzl)-L(-		29	720	1100
Ac-Pro-Leu-Ama(NHOH)-Trp-NMe_2		180	3700	1500
Ac-Pro-Leu-Ama(NHOH)-Tyr-L(-)MBA		46		
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-L(-	*)	3,5	4	
Ac-Pro-D-Leu-Ama(NHOH)-Tyr(Bzl)-L(-		750	12000	1500
Ac-D-Pro-D-Leu-Ama(NHOH)-Tyr(Bzl)-L(-		1000	10000	1200
Z-Pro-Leu-Ama(NHOH)-D-Tyr(Bzl)-			4500	2000
Z-Pro-Leu-Ama(NHOH)-D-Tyr(Bzl)-L(-		5000	6400	2000
Ac-Pro-Leu-Ama(N(Me)OH)-Tyr(Bzl)-L(-		1000		
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Leu-		0,7	28	21
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NHMe		0,25	49	12
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NHOH	*)	2	12	15
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NMe_2		0,3	9,8	6
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Phe-		0,8	35	40
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Val-		3		200
Ac-Pro-Leu-Ama(NHOH)-Tyr(Et)-NHMe		0,7	85	52
Ac-Pro-Leu-Ama(NHOH)-Tyr(Et)-NMe_2		1	32	17
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-L(-)MBA		2	140	70
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NHMe		4	140	
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NMe_2	*)	0,23	60	200
Boc-D/L-2-Pip-Leu-Ama(NHOH)-Tyr(Bzl)-		1500		
Bz-Ala-Ama(NHOH)-Tyr(Bzl)-NMe_2		1300		
Cp-CO-Leu-Ama(NHOH)-Tyr(Bzl)-L(-)MBA		140	>10000	9000

Abreviations:

2,4-DiMeP	2,4-dimethylpentylamine
Ac	acetyl
Ama	aminomalonic acid
Amp	p-aminophenylalanine
Bzl	benzyl
Bz	benzoyl
Cp	cyclopentane
Et	ethyl
MBA	methylbenzylamine
Me	methyl
Nal	β-naphthylalanine
Pip	pipecoline carboxylic acid
Z	benzyloxycarbonyl

natural amino acids are in three letter code

Table 3: Preliminary K_i-values in mole/l (not corrected)
Abreviations as above

Inhibitor	cdMMP-9	cdMT1-MMP	cdMMP-8
Boc-Ama(NHOH)-Tyr(Bzl)-L(-)MBA	$4,1x10^{-6}$	$3x10^{-5}$	
HCl H-Ama(NHOH)-Tyr(Bzl)-L(-)MBA	$9,1x10^{-6}$		
Boc-Ama(NHOH)-Tyr(Bzl)-NHOH	$9x10^{-7}$	$2x10^{-5}$	$3,5x10^{-6}$
Boc-Ama(NHOH)-Tyr(Bzl)-Val-Gly-NMe_2	$5x10^{-7}$	$2,5x10^{-6}$	$2,1x10^{-6}$

Table 4: Preliminary K_i-values in mole/l (not corrected)

Abreviations as above

Inhibitor	cdMMP-9	cdMT1-MMP	cdMMP-8
Ac-Pro-Leu-Ama(NHOH)OH	$1,4x10^{-4}$	$1x10^{-4}$	$1,2x10^{-5}$
Z-Pro-Leu-Ama$(NHOH)_2$	$4,2x10^{-5}$	$2,7x10^{-5}$	$3x10^{-6}$
Z-Pro-Ala-Ama$(NHOH)_2$	$3,1x10^{-5}$	$2,3x10^{-5}$	$1,8x10^{-5}$
Bz-Ala-Ama$(NHOH)_2$		$7x10^{-4}$	$3x10^{-4}$

Table 5: Preliminary K_i-values in nM (not corrected)

* structure see above

Inhibitor		cdMMP-2	cdMMP-13
Ac-Pro-Leu-Ama(NHOH)-Amp-L(-)MBA			
Ac-Pro-Leu-Ama(NHOH)-Amp(Bz)-L(-)MBA	*)		
Ac-Pro-Leu-Ama(NHOH)-Amp(CONHPh)-L(-			
Ac-Pro-Leu-Ama(NHOH)-Amp(Z)-L(-)MBA			
Ac-Pro-Leu-Ama(NHOH)-Leu-2,4-DiMeP			
Ac-Pro-Leu-Ama(NHOH)-2-Nal-NMe_2		190	35
Ac-Pro-Leu-Ama(NHOH)-D-2-Nal-NHMe			
Ac-Pro-Leu-Ama(NHOH)-D-2-Nal-NMe_2			
H-Pro-Leu-Ama(NHOH)-Phe-D(+)MBA			
H-Pro-Leu-Ama(NHOH)-Phe-L(-)MBA	*)		
Ac-Pro-Leu-Ama(NHOH)-Phe-Tyr(Bzl)-	*)		
Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-L(-	*)		
Ac-Pro-Leu-Ama(NHOH)-Ser(Bzl)-NMe_2	*)		
Ac-Pro-Leu-Ama(NHOH)-Thr(Bzl)-L(-			
Ac-Pro-Leu-Ama(NHOH)-Trp-NMe_2			290
Ac-Pro-Leu-Ama(NHOH)-Tyr-L(-)MBA			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-L(-	*)		
Ac-Pro-D-Leu-Ama(NHOH)-Tyr(Bzl)-L(-			
Ac-D-Pro-D-Leu-Ama(NHOH)-Tyr(Bzl)-L(-			
Z-Pro-Leu-Ama(NHOH)-D-Tyr(Bzl)-			
Z-Pro-Leu-Ama(NHOH)-D-Tyr(Bzl)-L(-			
Ac-Pro-Leu-Ama(N(Me)OH)-Tyr(Bzl)-L(-			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Leu-			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NHMe			13
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NHOH	*)		3
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-NMe_2			5,4
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Phe-			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Val-			3,8
Ac-Pro-Leu-Ama(NHOH)-Tyr(Et)-NHMe			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Et)-NMe_2		8,5	16
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-L(-)MBA			
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NHMe			20
Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NMe_2	*)	9	13
Boc-D/L-2-Pip-Leu-Ama(NHOH)-Tyr(Bzl)-			
Bz-Ala-Ama(NHOH)-Tyr(Bzl)-NMe_2			
Cp-CO-Leu-Ama(NHOH)-Tyr(Bzl)-L(-)MBA			

Materials and methods

The peptides containing an Ama(OMe) residue were synthesized by segment condensation of N-protected tripeptides of the type R_1-Pro-Aaa-Ama(OMe)OH and H-Tyr(Bzl)-R_2, where R_1 is a Boc protecting group or acetyl, Aaa is glycine, alanine or leucine and R_2 is an amide-bonded S(-)-α-methylbenzylamine, R(+)-α-methylbenzylamine or R(-)-α-methylcyclohexanemethylamine. Peptide coupling reactions were carried out in organic solvents following standard procedures (9). Alternatively an N-protected dipeptide Boc-Aaa-Ama(OMe)OH was coupled with the C-terminal fragment and, after removal of the Boc group, the R_1-Pro was added using an active ester. The conversion of the aminomalonic acid methylester function into the corresponding hydroxamic acid was effected via hydrolysis of the ester to the free malonic acid and treatment with hydroxylamine hydrochloride, a tertiary amine and DCC or directly by aminolysis of the methylester with hydroxylamine. Characterization of the synthesized inhibitors and precursors was carried out using NMR, liquid SIMS and ion spray mass spectrometry (9).

The inhibition constants K_i of the inhibitors were determined for the proteolytically activated form of native human gelatinase B (MMP-9) (10), the catalytic domain of neutrophil collagenase (cdMMP-8) (11), and for the additional enzymes of tables 2 to 5 given above using a spectrofluorometer Jasco FP-550 or a luminescence spectrometer Perkin-Elmer LS 50B. The substrate used, (7-methoxycumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-(2,4-dinitrophenyl)-L-2,3-diamino-propionyl)-Ala-Arg-NH_2 (12), is an internally quenched fluorescent peptide, which is cleaved at the Gly-Leu bond by the tested metalloproteinases. The resulting in-

crease of fluorescence was measured at an excitation wavelength of 328 nm and an emission wavelength of 393 nm.

The assay mixtures contained constant concentrations of the enzyme and 3% DMSO (as solvent for inhibitors and substrate) in 2 ml of buffer (0,1 M Tris-HCl pH 7.5, 0.1 M NaCl, 10 mM $CaCl_2$, 0.05% Brij 35). For at least three series with constant concentrations of substrate (approx. 0.2 to 1 µM) and different concentrations of the inhibitor every initial fluorescence increase, which is proportional to the substrate concentration and corresponds to the remaining proteolytic activity of the enzyme, was measured at 25°C. The substrate (predissolved 0.1 mg in 1 ml DMSO) was added after incubation of the enzyme with the inhibitor in the buffer for at least ten minutes. K_i values were determined by the established method of Dixon (13).

Activation of the latent progelatinase B (MMP-9), isolated from human neutrophils, was carried out in assay buffer by adding bovine trypsin (50 µl, 0.6 mg/ml) to the proenzyme (0.45 ml, 120 µg/ml) and incubating at 37°C for 10 minutes. The trypsin was then inactivated with aprotinin (50 µl, 1.2 mg/ml).

Examples

Preparation of Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Leu-NH_2.

Ac-Pro-Leu-OH.

30 mmole of each of Ac-Pro-OH, H-Leu-OMe hydrochloride, HOBT and N-ethylmorpholine are suspended in a mixture of 100 ml THF and 80 ml dichloromethane, and the resulting mixture is cooled to 0°C. Thereafter, 32 mmole of DCC are added in portions under

stirring for 2 h at 0°C, and stirring is continued at 5°C until completion of the reaction. After filtration the solution is evaporated and the residue taken into ethylacetate. The solution is than extracted sequentally with aqueous 10% NaCl/0,5 M HCl, 5% Na_2CO_3 and 5% $NaHCO_3$ solutions and is dried over sodium sulfate. After this, the solvent is removed in vaccuo. Finally, the residue is taken into a small volume dichloromethane, and the solution stored over night at 5°C and than filtered and evaporated.

Ac-Pro-Leu-OH.

20 mmole of Ac-Pro-Leu-OMe are dissolved in 40 ml methanol and added with 20 ml of aqueous 2 N NaOH at 0°C. After completion of the reaction it is acidified with a concentrated solution of $KHSO_4$, and the product is extracted with ethylacetate. Further purification can be conducted by additional extraction steps.

Ac-Pro-Leu-Ama(OMe)$_2$.

15 mmole of each of Ac-Pro-Leu-OH, aminomalonic acid dimethylester hydrochloride, HOBT and N-methylmorpholine are suspended in 100 ml of a mixture of THF, ethylacetate and dichloromethane (3:1:1). Thereafter, 15 mmole of DCC are added at 0°C, and it is stirred at 0-5°C until the reaction is nearly complete. The mixture is filtered, the solvent removed in vaccuo, and the residue taken into ethylacetate. The solution is extracted with 10% $NaHSO_4$, 5% Na_2CO_3 and 5% $NaHCO_3$ solutions and than dried over sodium sulfate. After this, the solvent is removed in vaccuo, and the residue taken into a small volume of dichloromethane. Finally, the solution is stored over night at 5°C and than filtered and evaporated.

Ac-Pro-Leu-Ama(OMe)OH.

10 mmole of Ac-Pro-Leu-Ama$(OMe)_2$ are completely dissolved in about 25 ml of absolute methanol, and under trapping any moisture and vigorous stirring at 0°C the solution is added dropwise with a solution of 9,5 mmole of KOH in about 10 ml of absolute methanol. The mixture is left stand over night at 0-5°C, optionally reduced in volume in vaccuo, and than under cooling with ice diluted with a 5% solution of $NaHCO_3$. After this, the aqueous solution is extracted several times with ethylacetate or a mixture of ethylacetate and diethylether and than slighly acidified under cooling with ice using concentrated $NaHSO_4$. The free malonic acid is extracted several times with etylacetate, and the combined organic phases are dried over sodium sulfate. Finally, the solvent is removed in vaccuo at max. 30°C.

Boc-Tyr(Bzl)-Leu-NH_2.

2 mmole of each of Boc-Tyr(Bzl)-OH, leucineamide hydrochloride, HOBT and N-ethylmorpholine are suspended in 10 ml THF. Than 1,05 mmole of EDC are added at 0°C, and it is stirred at 5°C until completion of the reaction. The reaction mixture is evaporated, the residue taken into ethylacetate and the solution purified by extraction.

H-Tyr(Bzl)-Leu-NH_2.

1,5 mmole of the Boc-protected derivatives are dissolved in about 6 ml of trifluoroethanol and added with 1 ml of concentrated HCl. After completion of the reaction 5% Na_2CO_3 solution is added and the product extracted with ethylacetate.

Ac-Pro-Leu-Ama(OMe)-Tyr(Bzl)-Leu-NH_2.

0,3 mmole of each of H-Tyr(Bzl)-Leu-NH_2 and HOBT and 0,33 mmole of Ac-Pro-Leu-Ama(OMe)OH are suspended in a mixture of 5 ml dichloromethane, 5 ml dimethoxyethane, 3 ml ethylacetate and 1 ml of methanol and added with a solution of 0,3 mmole of DCC in 2 ml dichloromethane at 0°C. The suspension is stirred at 5°C, added with 5 ml acetone and filtered, and the precipitate is recristallized several times from methanol.

Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Leu-NH_2.

0,1 mmole of the methylester are suspended in 3 ml of absolute methanol, and the mixture is added with hydroxylamine hydrochloride and, under trapping any moisture, at 0°C with sodium methanolate until the suspension reacts clearly alkaline. After completion of the reaction it is acidified with 10% aqueous $NaHSO_4$ solution and extracted with ethylacetate. The organic phases are dried over sodium sulfate and evaporated. Further purification can be performed by chromatographic methods.

Preparation of Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NMe_2.

Boc-Tyr(Me)-NMe_2.

The preparation is performed by condensation of Boc-Tyr(Me)-OH with dimethylamine similar to the synthesis of Boc-Tyr(Bzl)-Leu-NH_2.

H-Tyr(Me)-NMe_2 hydrochloride.

The preparation is performed by acidolysis of the Boc-protected derivate using a water-free saturated solution of HCl in acetic acid.

Ac-Pro-Leu-Ama(OMe)-Tyr(Me)-NMe_2.

0,25 mmole of H-Tyr(Me)-NMe_2 hydrochloride, 0,27 mmole of Ac-Pro-Leu-Ama(OMe)OH and 0,27 mmole of bromo-tris-(dimethylamino)-phosphonium-hexafluorophosphate are taken into 2,5 ml of water-free dichloromethane. While trapping any moisture and using a blanketing inert atmosphere, the mixture is added with 0,9 mmole diethylisopropylamine at 0°C and than stirred at 0-5°C until completion of the reaction. After this, the mixture is taken into ethylacetate and than extracted sequentally with aqueous 10% NaCl/0,5 M HCl, 5% Na_2CO_3 and 5% $NaHCO_3$ solutions and dried over sodium sulfate. Finally, the solvent is removed in vaccuo. The product is further purified chromatographically using Sephadex LH20 in methanol.

Ac-Pro-Leu-Ama(NHOH)-Tyr(Me)-NMe_2.

The preparation is analogous to the synthesis of Ac-Pro-Leu-Ama(NHOH)-Tyr(Bzl)-Leu-NH_2.

References

(1) Birkedal-Hansen, H., Moore, W.G.I., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A., Engler, J.A. (1993) Crit. Rev. Oral. Biol. Med. **4**, 197-250.

(2) Johnson, W.H., Roberts, N.A., Borkakoti, N. (1987) J. Enzyme Inhibition **2**, 1-22.

(3) Schwartz, M.A., Van Wart, H.E. (1992) Progress in Medicinal Chemistry **29**, 271-334.

(4) Bode, W., Reinemer, P., Huber, R., Kleine, T., Schnierer, S., Tschesche, H. (1992) EMBO J. **13**, 1263-1269.

(5) Grams, F., Crimmin, M., Hinnes, L., Huxley, P., Pieper, M., Tschesche, H., Bode, W. (1995) Biochemistry **34**, 14012-14020.

(6) Borkakoti, N., Winkler, F.K., Williams, D.H., D'Arcy, A., Broadhurst, M.J., Brown, P.A., Johnson, W.H., Murray, E.J. (1994) Struct. Biol. **1**, 106-110.

(7) Spurlino, J.C., Smallwood, A.M., Carlton, D.D., Banks, T.M., Vavra, K.J., Johnson, J.S., Cook, E.R., Falvo, J., Wahl, R.C., Pulvino, K.J., Wendoloski, J.J., Simth, D.L. (1994) Proteins Struct. Funct. Genet. **19**, 98-109.

(8) Schechter, I., Berger, A. (1967) Biochem. Biophys. Res. Commun. **27**, 157-162.

(9) Krumme, D. (1995) Diplomarbeit, Universität Bielefeld.

(10) Tschesche, H., Knäuper, V., Krämer, S., Michaelis, J., Oberhoff, R., Reinke, H. (1992) Matrix Supplement **1**, 245-255.

(11) Schnierer, S., Kleine, T., Gote, T., Hillemann, A., Knäuper, V., Tschesche, H. (1993) Biochem. Biophys. Res. Commun. **191**, 319-326.

(12) Knight, C.G., Willenbrock, F., Murphy, G. (1992) FEBS Lett. **296**, 263-266.

(13) Dixon, M. (1953) Biochem. J. **55**, 170-171.

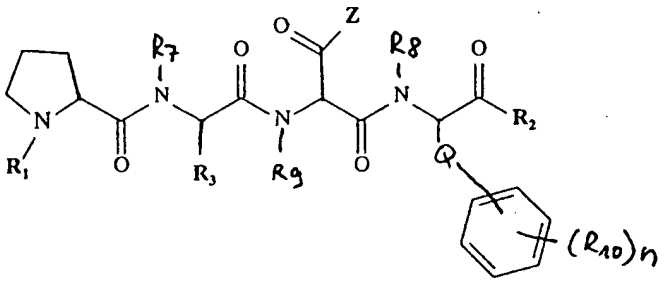
(14) Weingarten, H., Martin, R., Feder, J. (1985) Biochemistry **24**, 6730-6734.

(15) Fields, G.B., Van Wart, H.E., Birkedal-Hansen, H. (1973) J. Biol. Chem. **262**, 6221-6226.

(16) Netzel-Arnet, S., Fields, G., Birkedal-Hansen, H., Van Wart, H.E. (1991) J. Biol. Chem. **266**, 6747-6755.

Claims

1. Compounds containing aminomalonic acid derivatives and peptide backbone modified derivatives thereof of the general formulas I, II and III, IV, V and VI:



(I)

wherein

R_1 represents a N-protecting group like tert.butyloxycarbonyl, benzyloxycarbonyl or FMOC; or acetyl, -CO-lower alkyl, -CH_2-aryl, a natural amino acid, lower alkyl, aryl or H; or an optionally spacer linked: such as a synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

R_2 represents NH-D-C(Ph)-CH_3, NH-L-C(Ph)-CH_3, N(lower alkyl)$_2$, NH-lower alkyl, NH-aryl, a natural amino acid, a lower alkyl ester of an amino acid, O-lower alkyl, NHOH or OH, or an optionally spacer linked: such as a synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;
or R_4, wherein R_4 has the significance given below;

or the residue **Ccc**, wherein **Ccc** has the significance given below, optionally with a bounded residue R_2, wherein R_2 has the significance given earlier;
or **Z**, wherein **Z** has the significance given below;

R_3 represents lower alkyl or a side chain of a natural amino acid, or R_4;

R_7, R_8 **and** R_9 which may be the same or different from each other represent H, alkyl, aryl, OH, CO-lower alkyl, O-lower alkyl, O-CH_2-aryl, O-aryl, or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;
or an optionally spacer linked: such as synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

$(R_{10})_n$ (with n being selected from 0 to 5) which may be the same or different from each other are defined as R_2 or R_4 or $-(CH_2)_mR_4$ (with m being selected from 0 to 6);

R_4 represents H, alkyl, aryl, OH, O-lower alkyl, O-CH_2-aryl, O-aryl, NH-CO-aryl, NH-CO-NH-aryl, NH-CO-CH_2-aryl or NH-CO-R_5, wherein R_5 has the significance given below;
NH_2, NH-lower alkyl, N(lower alkyl)$_2$, N(lower alkyl 1)(lower alkyl 2), NHaryl, N(aryl)$_2$, N(aryl 1)(aryl 2), also combinations and pharmaceutically acceptable salts thereof;
N(lower alkyl)$_3^+$, N(aryl)$_3^+$, also combinations thereof;

or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;
or an optionally spacer linked: such as synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

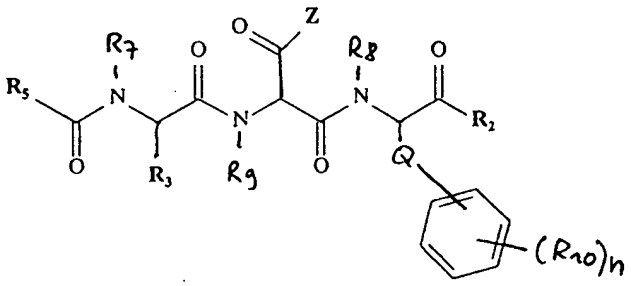
Z represents OH, O-lower alkyl, NHOH, $N(CH_3)OH$, $NHO-CH_3$, other NHO-lower alkyl,

Q represents $-(CH_2)_m-$, $-O-(CH_2)_m-$, $-CO-(CH_2)_m-$, $-(CH_2)_m-P-$, $-O-(CH_2)_m-P-$, $-CO-(CH_2)_m-P-$ (with m being selected from 0 to 6), wherein **P** represents cyclopropyl, cyclopentyl, cyclohexyl, 5- or 6-membered aryl, or a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and/or S as an additional ring member and (b) is optionally benzo-fused or optionally substituted on one or more other ring C-atoms by lower alkyl, aryl and/or oxo;
and pharmaceutically acceptable salts thereof; and

wherein
the term "lower alkyl", alone or in combination, means a straight-chain or branched-chain alkyl group containing a maximum of six carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert.butyl, n-pentyl, n-hexyl and the like;

the term "aryl" means phenyl, which is optionally substituted by lower alkyl, O-lower alkyl and/or halogen, i.e. fluorine, chlorine, bromine or iodine,

the term "spacer" means a straigth or branched alkyl-chain, amino alkyl-chain, carboxy alkyl-chain with a maximum length of 12 carbon atoms, or combinated forms, peptides or saccarides,



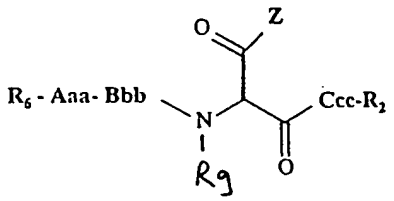
(II)

wherein R_2, R_3, R_7, R_8, R_9, R_{10}, Q, Z and n have the significance given earlier and

R_5 represents R_1-Proline, lower alkyl, aryl or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which is attached via the N-atom or via a C-atom and (a) optionally contains N, O and /or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo;

or an optionally spacer linked: synthetic or natural peptide, glycoprotein or the like, a solid or macromolecular product used for chromatographical procedures;

and pharmaceutically acceptable salts thereof;



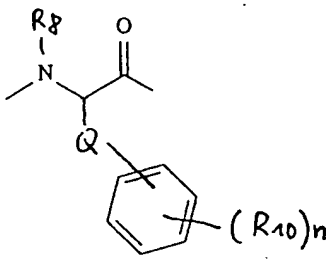
(III)

wherein R_2 , R_9 and Z have the significance given earlier and

Aaa represents a peptide bounded natural amino acid;

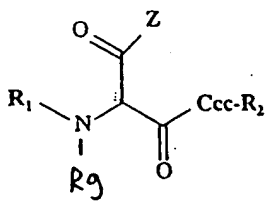
Bbb represents a peptide bounded natural amino acid;

Ccc represents a peptide bounded natural amino acid or Thr(Bzl),



Ser(Bzl) or the fragment or $NR_8C(QR_{10})HCO-$, wherein R_8, R_{10}, Q and n have the significance given earlier;

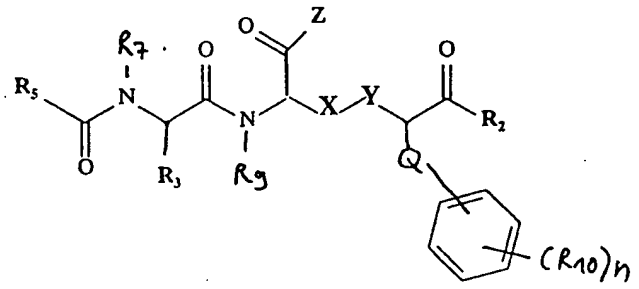
R_6 represents a N-protecting group, acetyl, -CO-alkyl(C_1-C_4), a natural amino acid, lower alkyl or H or R_1, wherein R_1 have the significance given earlier;
and pharmaceutically acceptable salts thereof;



(IV)

wherein R_1, R_2, R_9, Z and Ccc have the significance given earlier;

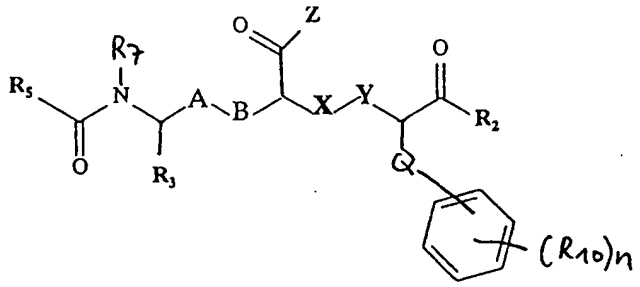
and pharmaceutically acceptable salts thereof;



V

wherein R_2, R_3, R_5, R_7, R_9, R_{10}, Q, Z and n have the significance given earlier,

X-Y represents especially CO-NH, CH_2-NH, CO-CH_2, CH_2-CH_2 CH_2-S, CH_2-O, CO-N(lower alkyl), CH_2-N(lower alkyl) or PHO_2-NH,
and pharmaceutically acceptable salts thereof;



VI

wherein R_2, R_3, R_5, R_7, R_{10}, Q, Z and n have the significance given earlier;

A-B and **X-Y** which may be the same or different from each other represent especially CO-NH, CH_2-NH, CO-CH_2, CH_2-CH_2 CH_2-S, CH_2-O, CO-N(lower alkyl), CH_2-N(lower alkyl)or PHO_2-NH;

and pharmaceutically acceptable salts thereof;
wherein any available hydrogen atom on any carbon or nitrogen atom in any of the above formulae I to VI and any of the corresponding substituents or groups as defined for said formulae may be in part or totally and independently from each other substituted by halogen (bromine, chlorine, fluorine or iodine), alkyl, especially lower alkyl, aryl, OH, CO-lower alkyl, O-lower alkyl, O-CH_2-aryl, O-aryl, or cyclopropyl, cyclopentyl, cyclohexyl, a 5- or 6-membered, aromatic or aliphatic N-heterocyclic ring which (a) optionally contains N, O and/or S as an additional ring member and (b) is optionally benz-fused or optionally substituted on one or more other C-atoms by lower alkyl, aryl and/or oxo.

2. Compounds according to claim 1, in the form of a L-enantiomer or a D-enantiomer or in the form of a diastereomer, including meso forms, or in the form of any mixture thereof, including racemic mixtures.

3. Use of compounds as defined in any preceeding claim as inhibitors of metalloproteases.

4. Use of compounds as defined in any preceeding claim as therapeutically active substances, especially in the control or prevention or in the treatment of:

degenerative joint diseases
rheumatoid arthritis
osteoarthritis
cancer
metastasis
tumour invasion
multiple sclerosis

parodontosis
fibrosis
Alzheimer´s desease
inflammatory bowel disease
neurodegenerative deseases
cerebral haemorrhage
wound healing
degenerative eye desease
aneurism
artificial joint replacement
organ transplantation
emphysema
Cholesteatom
Präeklampsie

5. Use of compounds as defined in any preceeding claim in chromatographical procedures.

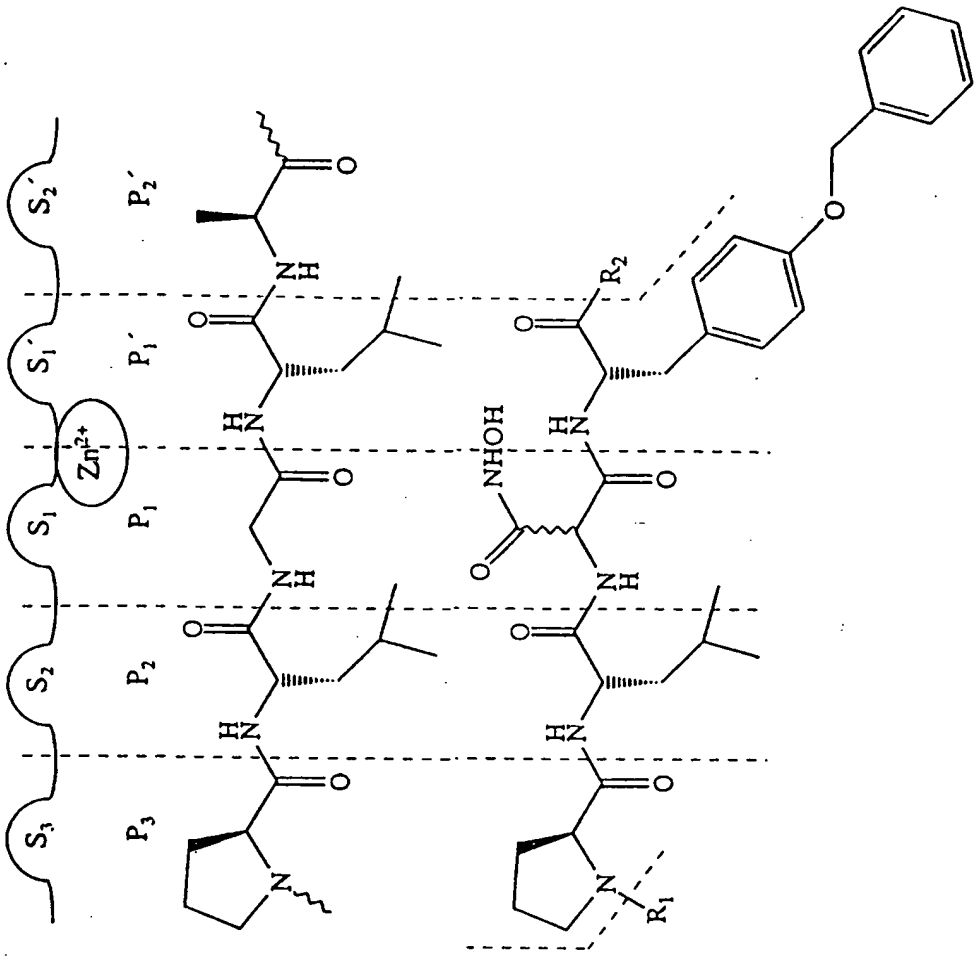

Subsites of the enzyme
Amino acid positions of :
Substrate
Inhibitor
S_3
S_2
S_1
S_1'
S_2'
P_3
P_2
P_1
P_1'
P_2'
Zn^{2+}
NHOH
R_1
R_2

Fig. 1

INTERNATIONAL SEARCH REPORT

Interr al Application No
PCT/EP 99/04826

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K5/117 C07K5/113 C07K7/06 C07K5/083 C07K5/097
C07K5/062 C07K5/072 A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KRUMME E.A.: "Hydroxamate derivatives of substrate-analogous peptides containing aminomalonic acid are potent inhibitors of matrix metalloproteinases" FEBS LETTERS, vol. 436, no. 2, 2 October 1998 (1998-10-02), pages 209-212, XP002120566 AMSTERDAM NL the whole document --- -/--	1-5

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 1999	10/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 99/04826

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CURTIN M L ET AL: "Broad spectrum matrix metalloproteinase inhibitors: an examination of succinamide hydroxamate inhibitors with P1Calpha gem -disubstitution" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 8, no. 12, 16 June 1998 (1998-06-16), page 1443-1448 XP004137061 ISSN: 0960-894X the whole document	1-5
A	GRAMS E.A.: "Structure determination and analysis of human neutrophil collagenase complexed with a hydroxamate inhibitor" BIOCHEMISTRY, vol. 34, no. 43, 1995, pages 14012-14020, XP002120567 EASTON, PA US cited in the application the whole document	1-5
A	YAMAMOTO E.A.: "Inhibition of membrane type 1 matrix metalloproteinse by hydroxamate inhibitors: an examination of the subsite pocket" JOURNAL OF MEDICINAL CHEMISTRY, vol. 41, no. 8, 9 April 1998 (1998-04-09), pages 1209-1217, XP002120568 WASHINGTON US the whole document	1-5

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/EP 99/04826

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 3 and 4 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-5(partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99 04826

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-5(partially)

Present claim 1 relates to an extremely large number of possible compounds. In fact, the claim contains so many options, variables and (mutual) references of variables, all relating to six different basic structures which actually do not share a significant structural invariable entity, that a lack of clarity and/or conciseness within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the complete subject-matter of said claim impossible.
Furthermore the available experimental data actually only comprise a very small part of the compounds claimed, which part is moreover not evenly distributed over the whole claimed area. Therefore claim 1 can also not be considered to represent a permissible generalisation which is fairly based on experimental evidence, that is, it is also not adequately supported by the description (Art.6 PCT).
Consequently, the search has been carried out for those parts of the application which do appear to be clear, concise and supported by the description, namely the compounds of the examples which are encompassed by claim 1, that is the compounds of the Tables 1-3 and 5 and their use as defined in the claims 3-5.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.